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# An Assessment of *In Vitro* Herbicidal Potential of Fungal Metabolites Against Parthenium Weed (*Parthenium hysterophorus* L.)

M A Halim Sheikh<sup>1</sup>, L Naher<sup>1,2\*</sup>, S M Rezaul Karim<sup>3</sup>, and P Sannasi<sup>1</sup> Norhafizah<sup>1</sup>

<sup>1</sup>Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia.

<sup>2</sup>Institute of food security and sustainable agriculture, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia.

<sup>3</sup>SHF Global Agrotech Ltd. Bangladesh.

E-mail: lailanaher@umk.edu.my

**Abstract.** Weed control by herbicide is issued for environmental problems and the emergence of resistance herbicide; thus, researchers are looking for alternative methods including bioherbicide. Hence, this study aims to isolate the pathogenic fungi that associated with parthenium weed (a significant noxious weed as a threat to agriculture) and then extract the fungal mycelia for application on seed germination inhibition to be potential as bioherbicide to control parthenium. In this study, the fungus of *Aspergillus sp.* and *Valsa mali* were isolated from parthenium leaf and their mycelium were extracted to isolate secondary metabolites using ethyl acetate solvent from the culture of potato dextrose broth (PDB) and malt extract broth (MEB) mediums. In vitro, both fungal metabolites were applied on seeds in plate assay experiment. Original and diluted culture filtrates of *Aspergillus sp.* inhibited the seed germination by 51% and 20% in PDB and 48% and 39% in MEB respectively, over control. Similarly, Original and diluted culture filtrates of *Valsa mali* significantly suppressed the seed germination by 52% and 24% in PDB and 62% and 33% in MEB respectively, over control. Therefore, it indicated that fermented culture mycelia metabolites from these fungi able to inhibit seed germination efficiently and can be potentially used as bioherbicide to control parthenium weed.

## 1. Introduction

Weeds are a significant problem for agricultural sector due to control strategy which involved plenty of labour works, using chemical herbicides that are costly, to ensure quality crop production and yield [1]. There are many weed in nature, among of all crop pests, parthenium weed (*Parthenium hysterophorus* L.) is a significant invasive weed that is troublesome for biodiversity and crop production globally [2]. It has gained tenth position among most noxious as devastating weeds in the world ranking [3, 4].

Many management options like cultural, mechanical and physical have been implemented to reduce crop losses by parthenium with inherent limitations in their application accordingly [5]. Chemical methods i.e. the application of herbicides is the utmost widely management tools, quick and effective in case of commercial crop production, but these options are limited due to creates herbicide resistance to the weeds, harmful for ecosystems [6]. It is costly, and leads to environmental pollution, potential health human risks, damage aquatic ecosystems, harm pollinators, affects the non-target beneficial microorganisms in the soil and it needed to be applied repeatedly by re-emerging capacity from their seed bank [7]. So, there are very limiting options for applying chemical approach to minimizing the economic losses and threatening agricultural sustainability [8]. Manipulation of



pathogens (microorganisms) and especially their natural products as herbicides have created momentous attention globally [9]. So, overcoming the unscrupulous application of chemical herbicides and world over emphasis towards adopting 'environment friendly' technologies to avoid the hazards of chemicals, non-biodegradable herbicides, considerable efforts have been made to apply alternative eco- friendly weed management technologies [10, 11]. Therefore, aim of this present research is to evaluate the effect of this toxic and noxious weed seed germination inhibition rate by using different fungal extracts metabolites namely *Aspergillus sp.* and *Valsa mali* which were isolated from infected parthenium weed.

## 2. Materials and Methods

### 2.1 Collection, symptoms, and isolations of disease samples

The study was conducted at Post-graduate laboratory, Faculty of Agro-based Industry, University Malaysia Kelantan, Jeli campus, during Dec' 2019 to March' 2020. The samples were collected from its infested state of Kedah, which is located in the northwestern part of Peninsular Malaysia. Diseased leaves with symptoms of necrotic, yellowish-brown colored tissues name as leaf blight disease and the reddish-brown name as cankers disease on the basal part. Then, the infected or disease showing parthenium leaves, stems and roots were collected and clean with hand brush to remove the plant debris and brought at UMK Jeli campus laboratory using in zippered plastic bags to isolate disease-causing pathogens.

### 2.2. Inoculated the samples and production of pure culture

The collected samples were surface sterilized with 10% sodium hypochlorite solution (Clorox, NaOCl) for 1-2 minutes, in 70% ethanol for 2 minutes. Then, the samples were wiped by sterilized tissue paper to dry up. Then, cleaned pieces sample were inoculated on PDA media contained in petridishes and incubated at room temperature for 3-4 days. Firstly, it was cultured on PDA for several times to obtain pure culture without any contamination of these fungi. Actively growing mycelium was transferred to a fresh PDA containing plate which was incubated for seven days to obtain a pure culture. Later, the pure culture was identified by using internal transcribe sequence (ITS) analysis.

### 2.3. Preparation of potato dextrose agar, potato dextrose broth (PDB) and malt extract broth (MEB)

The commercial potato dextrose agar (PDA) powder was used to culture the fungal isolate. Around 19.50 g of PDA powder was placed in an Erlenmeyer conical flask containing 500 ml of distilled water and the solution was mixed together and homogenate. After that, the mixture was autoclaved at 121°C for 15 minutes and then placed in the laboratory to cool down and about 9 ml of PDA media was poured in each petridish. The commercial PDB and MEB powder and same procedure was followed to prepare the culture media. Around 36 g each of PDB and MEB powder was placed in an Erlenmeyer conical flask containing 1.5 L of distilled water and the solution was mixed together and homogenate. Then, the mixture was autoclaved at 121°C for 15 minutes and placed in the laboratory to cool down and 100 ml of broth media was poured in each Erlenmeyer flask.

### 2.4. Preparation of fungal metabolites

The fungi grown on pure culture were transferred to 250 ml Erlenmeyer flasks that containing 100 ml of the two liquid growth mediums viz. potato dextrose broth (PDB) and malt extract broth (MEB) in separately. Eight plugs of each isolated fungus (7 days old) were cultivated in Erlenmeyer flask. The

pH of the medium was set to 5.8. All samples in the flasks were agitated using an orbital shaker at a speed of 120 rpm, 30°C. After 15 days of fermentation process, the broth which containing the fungal mycelia was filtered using a muslin cloth and filter paper (Whatman filter paper No. 1) prior to extraction process. This study used ethyl acetate for extraction of extracellular metabolites from the cultured fungi. The solvent of ethyl acetate and fermentation broth culture were mixed with a volume ratio of 1:1 in a separating funnel. After that, the solvent-broth combination was slowly shaken for 5 minutes and then used a rotary evaporator to evaporate the solvent only. The metabolites then kept in the refrigerator at 4°C for further use.

### 2.5. Determination and dilution procedure of the concentration of fungal metabolites

The fungal metabolites were taken from rotary evaporator and this volume extract refer as (100%) metabolites and 50% metabolites prepare as (v/v) by adding sterilized distilled water to obtain diluted fungal metabolites.

### 2.6. *Parthenium* weed seeds collection and viability test

The weed seeds with optimum maturity were obtained from the state of Kedah, Malaysia and collected the seed in brown paper envelopes to investigate the herbicidal effect of extracted fungal metabolites on parthenium seed germination. Seed viability test was carried out by placing of twenty seeds on moistened sterilized filter papers in each petri plate with thrice replications and it gave 95% germination. Plastic petri dishes (each of 9 cm diameter), per treatment were used, and then replicated three times. Petri dishes lined with absorbent cotton wool were rinsed with 3 ml of the different concentration treatments as 0% (water only served as control), 50% (metabolites diluted with distilled water) and 100% (original extraction without any dilution). The experiments were conducted in three replicates and each petri dish was moistened. The position of petri dishes was randomised weekly throughout the experiment in order to ensure equal distribution of sunlight and ensure more consistent coverage of water. The number of seeds that germinated was recorded daily for 20 days, and the percentage of seeds germinated was calculated. Germination inhibition percentage (IP) of treatments over the control germination was also calculated by using the following Equation 1-

$$IP = \frac{\text{Germinated seeds in control} - \text{Germinated seeds in metabolites}}{\text{Germinated seeds in control}} \times 100\% \quad (1)$$

### 2.7. Statistical Analysis

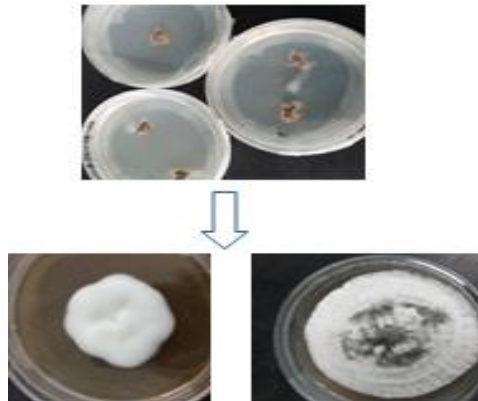
Standard errors of means were calculated. All statistical data were analyzed by Duncan's Multiple Range Test ( $P \leq 0.05$ ) using computer software SPSS at 5% level of significance [12] to analyze germination parameters.

## 3. Results

### 3.1 Fungal species

A total of five colonies were successfully isolated from the leaves tissue. There were no fungal colonies observed and isolated from root or basal tissues. The isolated colonies were further cultured on PDA media for pure culture. Apparently, two colonies were selected based on the morphological colour differences. Later, these isolations were sent for ITS analysis and the fungal colonies revealed as *Valsa mali* and *Aspergillus* sp. (the ITS data not shown here) in Figure 1. *Valsa mali*, is a pathogenic fungus for canker disease for apple fruit and no report found in another plant [13, 14]. There is no data found for *Valsa mali* fungus in Malaysian prospectus, which indicates it is a first

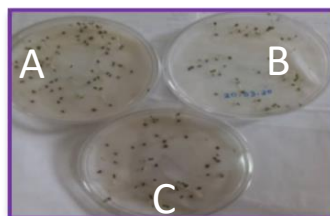
report in Malaysia. *Aspergillus* is ubiquitous fungus which can found in every agriculture and it has many important role either phytopathogen, decomposer or antibiotics. Recent study found it can use a remediation to degrade the chemical herbicide of glyphosate [15]. Hence, further study was evaluated on these two fungal species to inhibit seed germination to be a potential bioherbicide as both were isolated from parthenium plant.



**Figure 1.** Fungal isolation and pure culture on media. Sample inoculation plate (A); pure culture of *Valsa mali* (B); pure culture of *Aspergillus sp.* (C).

### 3.2 Seed Germination Bioassays

The study revealed that the metabolites of two fungal species showed the herbicidal activity on germination of seed in laboratory using two growth media viz. PDB and MEB. Original and diluted culture filtrates of *Aspergillus sp.* inhibited the seed germination by 51% and 20% in PDB and 48% and 39% in MEB respectively, over control (Table 1). Similarly, original and diluted culture filtrates of *Valsa mali* significantly suppressed the seed germination by 52% and 24% in PDB and 62% and 33% in MEB respectively, over control (Table 1). Original metabolites of *Aspergillus sp.* and *Valsa mali*, *P. hysterophorus* seed germination were inhibited by 48% and 62% in MEB medium respectively in petri dishes over control and diluted metabolites of *Aspergillus sp.* and *Valsa mali* were suppressed by 39% and 33% in MEB medium respectively in petri dishes over control. Likewise, original metabolites of *Aspergillus sp.* and *Valsa mali* were suppressed by 51% and 52% in PDB medium respectively in petri dishes over control and diluted metabolites of *Aspergillus sp.* and *Valsa mali* were inhibited by 20% and 24% in PDB medium respectively in petri dishes over control (Table 1).



**Figure 2.** Parthenium seeds germination inhibited in plate bioassay experiment. Control plate (A); seed treated by *Valsa mali* (B); seed treated by *Aspergillus sp.* (C).

Under the original extracts (100%) concentration of the fungal metabolites of both fungi significantly reduced the seed germination than the diluted (50%) one. On a control treatment with sterilized distilled water, no effect was found on seed germination. In this study, the fungal metabolites prepared both in MEB and PDB medium were exhibited greater herbicidal activity and more efficient in suppressing on germination of seeds in vitro.

#### 4. Discussion

Our study revealed that the metabolites of *Aspergillus* sp. and *Valsa mali* can suppress the phenological stage (seed germination) of *P. hysterophorus* particularly under higher concentrations. So, Metabolites of *Aspergillus* sp. proved the best effective herbicidal metabolites in laboratory bioassay. In the previous study, [16] revealed that herbicidal potential of metabolites of various phytopathogenic fungi namely *Ascochyta rabiei*, *Drechslera* spp., *Fusarium equiseti*, *Phoma glomerata* etc. against germination of alien parthenium weed and all the metabolites invariably suppressed the germination. In this study, the species of *Valsa mali* is newly reported as seed germination of parthenium weed. As stated earlier it is a pathogenic fungus for apple plant and apple fruit [14]. Thus, it can be informed that from this research the isolation of *Valsa mali* is first report in Malaysia. However, it is revealed that metabolites of *Aspergillus* sp. and *Valsa mali* have ability for inhibition of seed germination of *P. hysterophorus* which showed in our study. Nevertheless, further chemical identification of specific metabolites present in above fungi is essential to understand its efficacy.

**Table 1.** Effects of metabolites of two fungi prepared in PDB and MEB medium against germination of parthenium weed in laboratory bioassays

Media	Fungal species	Concentration (%)	Germination (%)	Inhibition (%)
Potato dextrose broth	<i>Aspergillus</i> sp.	50	76	20
		100	47	51
	<i>Valsa mali</i>	50	72	24
		100	46	52
Malt extract broth	<i>Aspergillus</i> sp.	50	58	39
		100	49	48
	<i>Valsa mali</i>	50	64	33
		100	36	62
Control		0	95	0

## 5. Conclusion

In this vitro study, two fungi were isolated from the infected parthenium weed which are *Aspergillus sp.* and *Valsa mali*. The results showed that *Aspergillus sp.* and *Valsa mali* both caused the toxic effect to inhibit parthenium seed germination. It could be noted there is no susceptible plant disease reported for *Valsa mali* fungus from Malaysia, so it also can be informed that this is the first report to isolate *Valsa mali* from infected parthenium weed in Malaysia. As reported in literature that *Valsa mali* is nonpathogenic fungus for other crops except Apple so this fungus has the prominent ability as bioherbicide to control parthenium weed in Malaysia. However, further study will be carried out to profile the effects on parthenium plants in quarantine house and subsequently in the field with these fungal metabolites.

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